

BIASES ASSOCIATED WITH DIET STUDY METHODS IN THE EURASIAN EAGLE-OWL

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ABSTRACT.—The diet of the Eurasian Eagle-Owl (*Bubo bubo*) was assessed by collecting pellets and prey remains near the nesting cliffs of 21 pairs in the central-eastern Italian Alps between 1993–97. Taxonomic and prey mass composition of the diet was compared between two methods of analysis, pellets and prey remains, to assess biases associated with these techniques. When compared with pellets, remains overestimated avian occurrence, underestimated mammals, and completely failed to detect fish occurrence ($P < 0.0001$). Large prey were also over-represented in remains ($P < 0.002$). Overall, pellets gave a more realistic and diverse picture of Eurasian Eagle-Owl diet, but failed to detect 26 avian species and 12 avian families identified in remains. Biases associated with the two methods may be lowered by pooling items collected by both methods, assuming the minimum possible number of individuals per species per collection event. However, care must be taken to show the relative contribution of each method in the pooled sample. Further research is needed to quantify biases in diet study methods, by using controlled feeding of captive owls. Similar biases may apply to the study methods commonly employed to assess the diet composition of other owls and predatory birds.

KEY WORDS: *bias; Bubo bubo; diet; diet assessment methods; Eurasian Eagle-Owl; Italy.*

Sesgos asociados con los métodos de estudio de dieta de *Bubo bubo*

RESUMEN.—La dieta de *Bubo bubo* fue evaluada mediante colecta de egagropilas y restos de presas cerca de las cornisas de anidación de 21 parejas en el centro oriente de los alpes italianos entre 1993–97. La composición taxonómica y la masa de las presas de la dieta fueron comparadas entre dos métodos de análisis en las egagropilas y los restos de presas, para evaluar los sesgos asociados con estas técnicas. Cuando fueron comparados con las egagropilas, los restos sobre estimaron la ocurrencia de las aves, subestimaron los mamíferos, y fallaron completamente en detectar la ocurrencia de peces ($P < 0.0001$). Las presas grandes además fueron sobre representadas en los restos ($P < 0.002$). En conjunto, las egagropilas dieron una imagen mas real y diversa de la dieta del búho, pero fallaron en detectar 26 especies de aves y 12 familias de aves identificadas en los restos. Los sesgos asociados con los dos métodos pueden ser disminuidos utilizando mancomunadamente los ítems colectados por ambos métodos, asumiendo el mínimo número posible de individuos por especie por evento de colección. Sin embargo, debe tenerse cuidado para la contribución relativa de cada método en la muestra mancomunada. Es necesaria mayor investigación para cuantificar los sesgos en los métodos de estudio de dieta, usando alimentación controlada en búhos cautivos. Sesgos similares pueden aplicar al estudio de métodos comunmente empleados para evaluar la composición de dieta de otros búhos y otras aves de presa.

[Traducción de César Márquez]

The diet of raptors has been assessed by analysis of stomach contents, pellets, or prey remains and by observation/photography of prey captured or

delivered to the nest (Marti 1987, Rosenberg and Cooper 1990). Biases associated with such methods have been examined in at least 11 species of diurnal raptors (Newton and Marquiss 1982, Collopy 1983, Goszczynski and Pilatowski 1986, Simmons et al. 1991, Bielefeldt et al. 1992, Mersmann et al. 1992, Mañosa 1994, Oro and Tella 1995, Real 1996, Sánchez-Zapata and Calvo 1998, Redpath et al.

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2001). Some common conclusions emerged from these studies: (1) remains usually overestimate the amount of large and conspicuous prey in the diet, such as large birds, large fish, or medium to large mammals; (2) pellets tend to overestimate the occurrence of medium to small prey, such as small mammals and passerine birds; and (3) when compared to direct-observation methods, remains seem to yield a more biased description of diet than pellets, but allow the detection of many unusual prey types not recorded in pellets, and the recognition of more items to the species level than pellets. Assessment of biases in diet study methods in nocturnal raptors presents additional problems, because of the difficulty of direct observation of prey capture or prey deliveries to the nest that usually occur at night.

The Eurasian Eagle-Owl (*Bubo bubo*) is a nocturnal top predator, with a generalist diet, locally specialized in medium-sized birds and mammals (Hiraldó et al. 1976, Donázar et al. 1989). Due to its frequent predation on other diurnal and nocturnal raptors, Eurasian Eagle-Owl populations can be a limiting factor for those of other birds of prey (Mikkola 1983, Sergio et al. 1999a, 1999b). The Eurasian Eagle-Owl diet has been extensively studied and recently reviewed (Mikkola 1983, Cramp 1985, Donázar et al. 1989, Penteriani 1996). Diet assessment has been carried out through analysis of pellets and/or remains, but no studies on biases associated with such methods have ever been published for this species. A correct evaluation of Eurasian Eagle-Owl diet is particularly important for two reasons: (1) to obtain a better understanding of its habitat use, diet composition, and conservation requirements; and (2) to obtain a more precise assessment of the impact of Eurasian Eagle-Owl predation on other raptors, or other conservation priority species. The aim of this work was to compare pellet contents with uneaten prey remains and to determine the most accurate method to assess the diet composition of this species. In particular we expected: (1) remains to overestimate large and conspicuous prey; (2) pellets to overestimate small items; and (3) the two methods to differ in their degree of taxonomic accuracy of prey identification.

STUDY AREA

Eurasian Eagle-Owls were surveyed in a 1330-km² study plot, located in the central-eastern Italian Alps (46°04'N, 11°08'E) (Marchesi et al. 1999). The area supported a population of 23–25 pairs. Elevation ranged from 70–

2400 m. The landscape was characterized by mountain slopes covered by broad-leaved woodland interspersed with extensive cliffs. The valley floors were intensively cultivated or urbanized.

METHODS

Pellets and prey remains were collected near the nesting cliffs of 21 pairs between 1993–97, by carefully searching the area near and under the nest, and at traditional roosting places. Collections were carried out at regular intervals throughout the year, so as to avoid biases caused by seasonal variations in the diet (Oro and Tella 1995). Prey items were identified by comparison to a reference collection at the Trento Natural History Museum. For each method and when pooling prey items based on both methods, items were identified assuming the minimum possible number of individuals per collection event. For example, if two individuals of a prey type were identified in pellets but only one individual was detected in remains from the same collection event, we registered two items to the pooled sample.

Prey mass was calculated based on information provided by Perrins (1987) and Macdonald and Barrett (1993). Eurasian Eagle-Owls usually capture juvenile individuals of prey species larger than a lagomorph (Donázar et al. 1989). Thus, half the mean adult mass of these species was employed, following Donázar et al. (1989). In addition, to avoid affecting the calculations of mean prey mass by few unusually heavy prey, no items were assigned a mass of more than 2500 g (ca. half the weight of an adult red fox, *Vulpes vulpes*, the heaviest local prey).

Statistical Analysis. Comparison of prey taxa between the two methods of diet assessment was performed by means of χ^2 analysis on contingency tables (Sokal and Rohlf 1981). To avoid cells with inadequate expected frequencies, prey items were grouped in the following categories: family Muridae, Gliridae, Erinaceidae, other mammals, Accipitridae, Strigidae, Phasianidae, Columbidae, Rallidae, Turdidae, Corvidae, other birds, unidentified birds, and fish. Hereafter, we refer to such groups as “main prey categories.”

Mean prey mass was compared between methods by means of *t*-tests (Sokal and Rohlf 1981). Mean number of species identified per detected family was compared between methods with Matched Pairs *t*-tests (Sokal and Rohlf 1981). Dietary breadth within each diet analysis-method was estimated through the Simpson's index, calculated as $\sum p_i^2$, where p_i is the relative proportion of each prey category within the sample (Simpson 1949). The index ranges between 0–1, with higher values indicating lower diet diversity. Dietary overlap between different methods was estimated through the Pianka's index, ranging from zero (no overlap) to one (complete overlap; Pianka 1973). When comparing pellets and remains, different analyses were carried out for birds, mammals, and overall vertebrates composition to gain further insights into differences between the methods. When multiple comparisons were carried out on the same data set, the sequential Bonferroni correction was used to adjust the significance level (Rice 1989). Means are given with 1 SE, all tests are two-tailed, and statistical significance was set at $P < 0.05$.

Table 1. Percentage frequency of main prey categories of Eurasian Eagle-Owls in the Italian Alps (1993–97), as determined by two methods of diet analysis and by the combination of both methods (pooled).

PREY CATEGORY	PELLETS		REMAINS		POOLED ^a	
	N (%)	% MASS	N (%)	% MASS	N (%)	% MASS
Mammals:	461 (76)	83.1	53 (28.2)	45.4	493 (65.6)	69.9
Muridae	203 (33.5)	29.3	0 (0.0)	0.0	203 (27.0)	21.2
Gliridae	173 (28.5)	11.9	4 (2.1)	0.5	175 (23.3)	8.7
Erinaceidae	63 (10.4)	25.8	45 (23.9)	35.2	92 (12.3)	27.3
Other mammals ^b	22 (3.6)	16.2	4 (2.1)	9.7	23 (3.1)	12.6
Birds:	130 (21.5)	13.4	135 (71.8)	54.6	243 (32.3)	28.4
Accipitridae	9 (1.5)	1.9	9 (4.8)	4.5	15 (2.0)	2.6
Strigidae	5 (0.8)	0.9	16 (8.5)	4.9	18 (2.4)	2.1
Phasianidae	5 (0.8)	4.2	9 (4.8)	11.3	12 (1.6)	6.2
Columbidae	0 (0.0)	0.0	24 (12.8)	8.7	24 (3.2)	3.4
Rallidae	0 (0.0)	0.0	7 (3.7)	2.0	7 (0.9)	0.8
Turdidae	85 (14.0)	4.4	23 (12.2)	2.2	96 (12.8)	3.6
Corvidae	5 (0.8)	2.0	26 (13.8)	13.6	29 (3.9)	6.1
Other birds ^c	3 (0.5)	1.1	18 (9.6)	7.4	21 (2.8)	3.7
Unidentified birds	18 (3.0)		3 (1.6)		21 (2.8)	
Fish	15 (2.5)	2.3	0 (0.0)	0.0	15 (2.0)	1.7
Total	606		188		751	
Simpson's index	0.23	0.22	0.13	0.18	0.16	0.16

^a Calculated by pooling pellets and prey remains and assuming the smallest possible number of individuals per prey species per collection event.

^b Includes families Talpidae, Canidae, Felidae, and Leporidae.

^c Includes families Podicipedidae, Ardeidae, Anatidae, Falconidae, Tetraonidae, Laridae, Cuculidae, Apodidae, Picidae, Hirundinidae, Sturnidae, and Laniidae.

RESULTS

Comparison of Pellets and Prey Remains.

All vertebrates. Pellets and prey remains differed significantly in frequency of mammals, birds, and fish recorded ($\chi^2_2 = 194$, $P < 0.0001$; Table 1). Frequency of main prey categories differed between the two methods ($\chi^2_{13} = 421$, $P < 0.0001$; Table 1). Twelve species and six families recorded in pellets went undetected in remains. Twenty-six species and 12 families found in remains were not recorded in pellets. Of the main prey categories, two were undetected in pellets and two in remains (Table 1). Fewer items were identified at the species level in pellets than in remains (76% and 98%; $\chi^2_1 = 76$, $P < 0.0001$). Mean number of identified species per detected family did not vary significantly between methods (0.97 ± 0.2 and 1.37 ± 0.3 , $t_{30} = -1.44$, $P = 0.16$). Mean prey mass was significantly lower in pellets than in remains (332 ± 16 and 552 ± 37 , respectively; $t_{588,185} = -5.3$, $P < 0.002$; Fig. 1). Diet diversity was higher in re-

main than in pellets (Table 1). Pianka's index of overlap was 0.30 by number and 0.59 by mass.

Mammals. Frequency occurrence of taxonomic groups significantly differed between pellets and remains ($\chi^2_3 = 134$, $P < 0.0001$; Table 1). Nine species, four families and one main prey category recorded in pellets went completely undetected in remains, while all species, families, and main prey categories identified in remains were represented in pellets (Table 1). A lower frequency of items was identified at the species level in pellets than in remains (78% and 91%; $\chi^2_1 = 20$, $P = 0.0001$). Mean number of identified species per detected family was higher in pellets than in remains (1.56 ± 0.3 and 0.56 ± 0.2 , respectively; $t_9 = 3.00$, $P = 0.034$). Mean prey mass was significantly lower in pellets than in remains (333 ± 17 and 534 ± 34 , respectively; $t_{573,200} = -5.3$, $P < 0.003$). Dietary breadth was higher in pellets than in remains: the Simpson index was 0.36 in pellets and 0.73 in remains. Pianka's index of overlap between the two methods was 0.29 by number and 0.68 by mass.

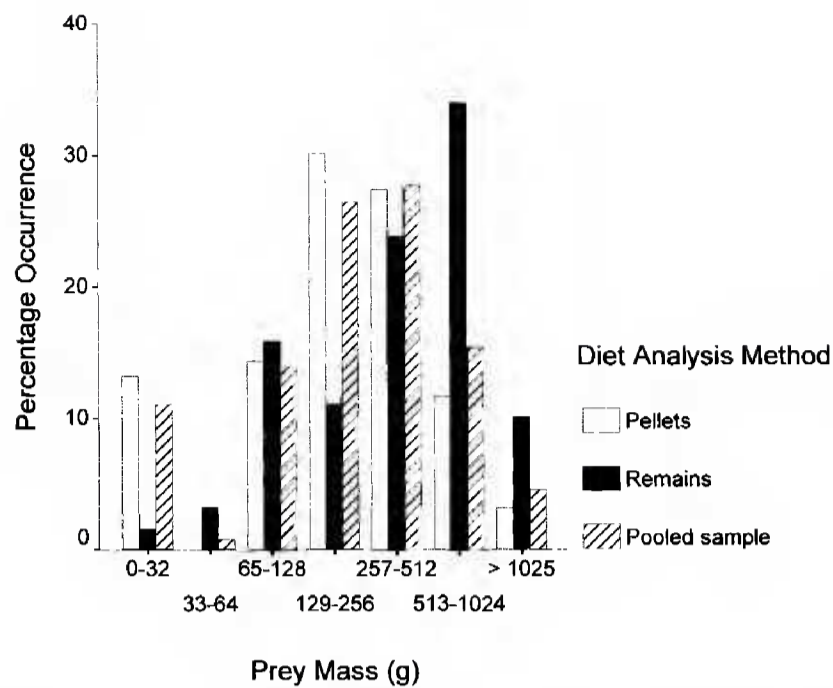


Figure 1. Mass distribution of prey captured by Eurasian Eagle-Owls in the central-eastern Italian Alps (1993–97), as estimated by pellet analysis, remains analysis, or by pooling pellets and remains. Prey items were grouped by mass categories using an exponential distribution in base two.

Birds. Taxonomic composition significantly differed between pellets and remains ($\chi^2_8 = 127$, $P < 0.0001$; Table 1). Twenty-six species and 12 families identified in remains went completely undetected in pellets, while only three species and one family identified through pellet analysis were not detected in remains. Two main prey categories went unrecorded in pellets, but were common in remains (Table 1). A higher percentage of items were identified at the species level in remains than in pellets (82% and 2%, respectively; $\chi^2_1 = 206$, $P < 0.0001$; Table 1). The mean number of identified species per detected family was significantly higher in remains than in pellets (1.89 ± 0.3 and 0.68 ± 0.3 , respectively; $t_{19} = -4.46$, $P < 0.003$). Mean avian prey mass was significantly higher in remains than in pellets (423 ± 38 and 254 ± 37 , respectively; $t_{132,112} = -3.1$, $P = 0.002$). The Simpson's index was 0.57 in pellets and 0.13 in remains, indicating a more diverse diet in the latter method. Pianka's index of overlap between the two methods was 0.56 by number and 0.69 by mass.

Comparison of the Pooled Sample with Pellets and Remains. All species, families, and main prey categories recorded in pellets or remains were obviously detected in the pooled sample. A higher percentage of items were identified at the species level in the pooled sample than in pellets (81% and 76%, respectively; $\chi^2_1 = 5$, $P = 0.019$) and in

remains than in the pooled sample (98% and 81%, respectively; $\chi^2_1 = 49$, $P < 0.0001$). Mean number of identified species per detected family was higher in the pooled sample than in pellets (1.77 ± 0.2 and 0.97 ± 0.2 , respectively; $t_{30} = -4.00$, $P < 0.001$) and remains (1.37 ± 0.2 , $t_{30} = 3.29$, $P = 0.003$). Mean prey mass did not differ between the pooled sample and pellets (368 ± 15 and 332 ± 16 , $t_{730,588} = -1.62$, $P = 0.12$; Fig. 1) and was significantly lower in the pooled sample than in remains (552 ± 37 ; $t_{730,185} = 5.21$, $P < 0.001$). Diet diversity in the pooled sample was intermediate between that in pellets and remains (Table 1). Overlap between the pooled sample and pellets was 0.99 by number and 0.94 by mass. Overlap between the pooled sample and remains was 0.46 by number and 0.79 by mass.

DISCUSSION

The direct observation of prey capture or delivery to the nest is considered the least biased method of diet analysis (Simmons et al. 1991, Bielefeldt et al. 1992). However, this method is very time-consuming, often unfeasible for many species, and particularly poorly suited to the study of Eurasian Eagle-Owl diet, because of this species' nocturnal habits and generally inaccessible cliff nest sites. Indirect methods, such as analysis of pellets and remains, are thus required. Due to the above difficulties, we were unable to compare pellet and remains analyses with direct observation of prey delivered to the nest. However, comparison of the two indirect methods suggested that both of them incorporated inherent biases.

Overlap in frequency of main prey categories between the two methods was extremely low. When compared to pellets, remains overestimated birds, underestimated mammals, and failed to detect the presence of fish in the diet. Large prey were also overrepresented in remains when compared to pellets. Biases in remains toward underestimation of fish occurrence and overestimation of bird occurrence and of large prey have been detected in other studies which compared remains with direct observations (Simmons et al. 1991, Bielefeldt et al. 1992, Mersmann et al. 1992, Real 1996). Such biases are probably caused by the different conspicuousness and rates of deterioration of the different body parts of different taxa (Goszczyński and Pilatowski 1986, Mersmann et al. 1992). For example, avian pluckings are generally more colorful and conspicuous than other vertebrates' remains

(Bielefeldt et al. 1992); in addition, pluckings of large birds are generally more conspicuous and characterized by lower decay rates than those of smaller birds (Goszczyński and Pilatowski 1986, Newton and Marquiss 1982). Overall, remains generally consisted of large, easily-identifiable body parts. As a result, they were more frequently identifiable to the species level than prey items in pellets.

Comparison of pellets and remains within different vertebrates' prey groups showed biases to be affected by an interaction between different methods of analysis and different prey taxa. Within mammals, remains failed to detect the presence of the whole family Muridae, which accounted for 33.5% of the items in pellets. Remains were strongly dominated by the conspicuous, large skins of hedgehogs (*Erinaceus europaeus*). Within birds, pellets failed to record the presence of 26 species and 12 families identified in remains. Thus, remains gave a more complete and diverse picture of avian diet, but still overestimated prey size. Finally, fish were recorded only in pellets. Thus, pellets seemed to yield a more balanced and realistic picture of Eurasian Eagle-Owl diet, but remains seemed to be a useful complementary tool to assess avian occurrence. As an additional advantage, pellets yielded a much higher number of prey items than remains, despite equal search effort by the researchers. Pellets were thus a more cost-effective method to collect large numbers of prey.

Research Implications. Because of the difficulty of direct-observation methods and the biases inherent in pellets and remains analyses, Eurasian Eagle-Owl diet should be assessed through multiple techniques, including the pooling of samples derived from different methods of analysis. In our study, all species and families identified in pellets and remains were represented in the pooled sample. When compared to pellets and remains, the pooled sample showed an intermediate mean prey mass, diet diversity, and frequency of items identified at the species level. Because of the different sample sizes of pellets and remains, the overlap was high between the pooled sample and pellets, and less so between the pooled sample and remains. By reflecting more the taxonomic and prey-mass composition of pellets and by adding to it the additional avian prey detected through remains, the pooled sample lowered biases associated with each method and provided additional insights in Eurasian Eagle-Owl diet composition. The use of pooled samples

consisting of pellets and remains has been proposed for other raptors (Goszczyński and Pilatowski 1986, Simmons et al. 1991, Mañosa 1994, Oro and Tella 1995) and has been demonstrated to yield a relatively close fit to diet composition assessed by direct observation (Simmons et al. 1991, Mañosa 1994, but see Redpath et al. 2001).

Further research is needed to assess precise biases associated with different methods of diet analysis by means of controlled feeding of captive Eurasian Eagle-Owls, as carried out for other species (Mersmann et al. 1992, Real 1996). In the absence of such data, we suggest that future studies of Eurasian Eagle-Owl diet be carried out by using multiple techniques in a complementary way. Pooling samples obtained through pellet and remains can reduce biases, but care should be taken to show the relative contribution of each method to the pooled sample in terms of taxonomic and prey mass composition (Table 1). In the past, pooled samples have been presented without specifying the relative contribution of pellet items and remains to the overall sample (e.g., Olsson 1979, Hirald et al. 1975). Such inaccuracy can produce biases in different studies, making them difficult to compare and adding statistical noise to review analyses of diet composition (Donazar et al. 1989). Review studies on predation rate of Eurasian Eagle-Owls on raptors or other conservation sensitive species (e.g., Serrano 2000) should take into account biases inherent to different diet-analysis techniques: studies based exclusively on remains or pellets are likely to overestimate or underestimate, respectively, Eurasian Eagle-Owl impact on other large avian species. Such review analyses should be carried out ideally on pooled samples and rerun for each diet analysis technique separately, to compare their results. Finally, as similar biases are likely to apply to many other species, we suggest that similar approaches to diet assessment be used on other owls or other avian predators.

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